IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application(s) of:

Ratledge, Colin et al.

Serial No.: unknown 10/030700
Filed: unknown

For: Currently pending unpublished application(s) with any one of the parent applications named below

Parent Patent Application:

PCT/GB00/02695
(filed 13 July 2000) (International Publication No. WO 01/04338)

Title: "Culture of *Crypthecodinium cohnii* for the Synthesis of a Polyunsaturated Fatty Acid"

Group Art Unit:

Examiner:

"EXPRESS MAIL" MAILING LABEL NUMBER: EV493476348US

DATE OF DEPOSIT: January 28, 2005

I HEREBY CERTIFY THAT THIS PAPER OR FEE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE VIA "EXPRESS MAIL" ADDRESSED TO THE COMMISSIONER FOR PATENTS, P.O. BOX 1450, ALEXANDRIA, VA.22313-1450.

TYPED OR PRINTED NAME: Chasity C. Rossum

SIGNATURE: Chasity Rossum

OK to scan

WILLIAM R. DIXON, JR.
SPECIAL PROGRAM EXAMINER
7/6/05

Office of Petitions
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

PROTEST UNDER 37 C.F.R. § 1.291(a)

The undersigned, having reviewed the specification and the claims of the parent application of the above-identified patent application(s), and having formed the opinion that the subject matter of said application(s) is not patentable in view of certain prior art references and other information, and as a consequence thereof being further of the opinion that the grant by the United States Patent and Trademark Office of a patent to the applicants of the above-identified patent application(s) would be contrary to the public interest, does hereby protest the application(s) pursuant to 37 C.F.R. § 1.291(a). We are enclosing this Protest in duplicate as service is not possible. Additionally, as Protestor is unable to specifically identify the U.S. Serial Number of the application, this Protest is being directed towards the Office of Petitions as directed by MPEP 1901.03.

It is believed that there may be an unpublished pending patent application with the above-mentioned parent international (PCT) application. The above-mentioned international application was filed on July 13, 2000. The Patent Office publication of patent application provisions apply to

international applications filed on or after November 29, 2000, before the date of the above-mentioned international application. See U.S. Patent Office Web Publication, 18-month Publication of Patent Applications, '[t]he Eighteen-Month Publication of Patent Applications provisions apply to applications (other than for a design patent) filed under 35 United States Code (U.S.C.) 111(a) on or after November 29, 2000, and to applications in compliance with 35 U.S.C. 371 that resulted from international applications filed under 35 U.S.C. 363 on or after November 29, 2000." Accordingly as the publication provisions apply to applications filed on or after November 29, 2000, the present U.S. application, if existent, with an international filing date of July 13, 2000, would not be published. Accordingly, this document is Filed in Accordance with 37 C.F.R. § 1.291(a), "Protests by the Public Against Pending Applications."

Attached hereto in accordance with 37 C.F.R. § 1.291(a) are: (1) a listing on the accompanying form PTO-1449 of the patents and publications relied upon; (2) a concise explanation of the relevance of each of the listed items; and (3) a copy of each listed patent or publication in written form. It is believed that this Protest is being filed before the mailing of a notice of allowance under 37 C.F.R. § 1.311.

The Subject Matter of the Application

The application(s) identified above (having parent application PCT/GB00/02695 (filed 13 July 2000, International Publication No. WO 01/04338) are directed to methods of culturing a microorganism for the synthesis of polyunsaturated fatty acid, especially docosahexaenoic acid, comprising culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group, the microorganism using the species as a carbon source and synthesizing polyunsaturated fatty acid, especially docosahexaenoic acid. This protest is being submitted against this subject matter.

Subject Matter that is Subject to this Protest

For convenience, reference will be made to the following subject matter:

Embodiment A–a method of culturing a microorganism for the synthesis of docosahexaenoic acid by the microorganism, comprising culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group, the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid.

Embodiment A(1)–the method of Embodiment A, wherein the organism is a dinoflagellate or genetically modified variant thereof.

Embodiment A(2)–the method of Embodiment A(1), wherein the microorganism is *Crypthecodinium cohnii* or a genetically modified variant thereof.

Embodiment A(3)–the method of Embodiment A, wherein the species is a carboxylic acid or carboxylate ion.

Embodiment A(4)–the method of Embodiment A, wherein the species is acetic acid or acetate.

Embodiment A(5)–the method of Embodiment A, wherein the species is the main carbon source for the microorganism during the culture of the microorganism.

Embodiment A(6)–the method of Embodiment A, wherein the microorganism is cultured in a medium, an amount of the species being provided in the medium over a period of time during the culture of the microorganism.

Embodiment A(7)–the method of Embodiment A(6), wherein the use of the species as a carbon source by the microorganism causes an increase in pH of the medium, said provision of the species comprising addition of an organic acid to the medium in response to the increase in pH so as to decrease the pH of the medium.

Embodiment A(8)–the method of Embodiment A(7), wherein the organic acid is the species.

Embodiment A(9)–the method of Embodiment A(7), wherein the organic acid ionizes to form the species.

Embodiment A(10)–the method of Embodiment A(7), wherein the organic acid is added so as to maintain the pH substantially at a desired value.

Embodiment A(11)–the method of Embodiment A(7), wherein the desired value is pH 6.5.

Embodiment A(12)–the method of Embodiment A(7), wherein the pH of the medium is monitored by means that produces a signal that is used to control the addition of the organic acid to the medium.

Embodiment A(13)–the method of Embodiment A(12), wherein signal is used to control addition of one or more of a nitrogen source, a phosphorous source, an amino acid, a vitamin, a salt or another growth factor during the culture of the microorganism.

Embodiment A(14)–the method of Embodiment A(7), wherein the organic acid is added to the medium in a mixture comprising a further compound.

Embodiment A(15)–the method of Embodiment A(14), wherein the further compound is a further organic acid.

Embodiment A(16)–the method of Embodiment A(14), wherein the further compound is a lipid.

Embodiment A(17)–the method of Embodiment A(14), wherein the mixture is a waste product from an industrial process

Embodiment A(18)–the method of Embodiment A(14), wherein the further compound is a nitrogen source, a phosphorus source, an amino acid, a vitamin, a growth factor, a salt or a lipid.

Embodiment A(19)–the method of Embodiment A, wherein prior to said culturing with said species, the microorganism is grown with said species.

Embodiment A(20)–the method of Embodiment A, wherein the microorganism is cultured with an organic nitrogen source, preferably with yeast extract.

Embodiment A(21)–the method of Embodiment A(20), wherein the nitrogen source is yeast extract and the initial concentration of the yeast extract is greater than 7.5 g/l.

Embodiment A(22)–the method of Embodiment A(20), wherein the initial concentration of yeast extract is 10 g/l.

Embodiment A(23)–the method of Embodiment A, wherein the microorganism is cultured with salts or osmoticants, preferably with sea salts.

Embodiment A(24)–the method of Embodiment A, wherein said culture is performed as a batch process or a fed-batch process.

Embodiment A(25)–the method of Embodiment A(24), wherein the culture is performed for between about 4 to 10 days, preferably between about 6 to about 9 days.

Embodiment A(26)–the method of Embodiment A, wherein said culture is performed as a continuous process or a semi-continuous process.

Embodiment A(27)–the method of Embodiment A, wherein the method further comprises extracting oil including docosahexaenoic acid from the microorganism and, preferably, purifying the oil to increase the docosahexaenoic acid content of the oil.

Embodiment A(28)–the method of Embodiment A, wherein the method further comprises the purification or partial purification of docosahexaenoic acid from the microorganism.

Embodiment A(29)–the method of Embodiment A, wherein the culture does not include a stationary phase.

Embodiment A(30)–an oil comprising docosahexaenoic acid prepared from a microorganism cultured in accordance with Embodiment A.

Embodiment A(31)–an at least partially purified preparation of docosahexaenoic acid prepared from a microorganism cultured in accordance with Embodiment A.

Embodiment A(32)–the method of Embodiment A(4), wherein the initial concentration of the species is about 8 g/l.

Embodiment B–a method of culturing a microorganism for the synthesis of docosahexaenoic acid by the microorganism, comprising culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group, the microorganism sythesizing docosahexaenoic acid containing carbon from the species.

Embodiment C–a method of culturing a microorganism for the synthesis of a polyunsaturated fatty acid by the microorganism, comprising culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group, the microorganism using the species as a carbon source and synthesizing a polyunsaturated fatty acid.

Embodiment C(1)–an oil comprising the polyunsaturated fatty acid of Embodiment C, prepared from a microorganism cultured in accordance with Embodiment C.

Embodiment C(2)–an at least partially purified preparation of the polyunsaturated fatty acid of Embodiment C, prepared from a microorganism cultured in accordance with Embodiment C.

Embodiment C(3)–a method comprising using a microorganism according to Embodiment C as a food source.

Embodiment D–a microorganism cultured in accordance with any one of Embodiments A, B, or C.

The References

Reference is made to the following documents:

EP 0 515 460	12/2/1992	Europe	A23L	1/054
US 5,130,242	7/14/1992	Barclay	435	134
WO 89/00606	1/26/1989	PCT	C12P	7/64

Bahnweg; "Studies on the Physiology of Thraustochytriales, II. Carbon Nutrition of Thraustochytrium spp., Schizochytrium sp., Japanochytrium sp., Ulkenia spp., and Labyrinthuloides spp."; *Veroff. Inst. Meeresforsch. Bremerh*; (1979); 17:pp. 269-273

Bajpai et al.; "Production of Docosahexaenoic Acid by Thraustochytrium Aureum"; *Applied Microbiology and Biotechnology*; (1991); 35: pp. 706-710

Beach et al.; "Biosynthesis of Oleic Acid and Docosahexaenoic Acid by a Heterotrophic Marine Dinoflagellate Crypthecodinium Cohnii"; *Biochimica et Biophysica Acta*; (1974); 369: pp. 16-24

Droop; "Algal Physiology and Biochemistry, Chapter 19: Heterotrophy of Carbon"; *University of California Press*; (1974); Vol. 10; pp. 530-559

Gold et al.; "Growth Requirements of Gyrodinium cohnii"; *J. Protozool.*; (1966); Vol. 13, No. 2; pp. 255-257

Hastings et al.; "Qualitative Requirements and Utilization of Nutrients: Algae"; *Handbook Series in Nutrition and Food, Section D: Nutritional Requirements, CRC Press, Cleveland, Ohio*; (1977); Vol. I; pp. 87-163

Henderson et al.; "Lipid Composition and Biosynthesis in the Marine Dinoflagellate Crypthecodinium Cohnii"; *Phytochemistry*; (1988); Vol. 27, No. 6; pp. 1679-1683

Henderson et al.; "Polyunsaturated Fatty Acid Metabolism in the Marine Dinoflagellate Crypthecodinium Cohnii"; *Biochemistry*; (1991); Vol. 30, No. 6; pp. 1781-1787

Provasoli; "Marine Ecology, 5. Cultivation of Animals"; *John Wiley & Sons*; (1977); Vol. 3; pp. 1295-1319

Provasoli et al.; "Nutrition of the American Strain of Gyrodinium cohnii"; *Archiv fur Mikrobiologie*; 42; pp. 196-203

Anticipation–35 U.S.C. 102

Anticipation under 35 U.S.C. § 102 occurs when "each and every element as set forth in the claim, either expressly or inherently described, in a single prior art reference." See Verdegaal Bros. v. Union Oil Co. of Ca., 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). Additionally, under the principle of inherency, when a claim recites using an old composition or structure and the "use" is directed to a result or property of that composition or structure, then that claim is anticipated. See MPEP 2112.02 (citing In re May, 574 F.2d 1082, 1090, 197 USPQ 601, 607 (CCPA 1978)).

Embodiment A. Embodiment A refers to a method of culturing a microorganism for the synthesis of docosahexaenoic acid by the microorganism, comprising culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group, the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid. The following references contain all of the elements of Embodiment A (as described more specifically below) and are therefore anticipatory references.

I. Gold and Baren

This reference discloses "culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group." Gold and Baren teach the use of 1500 mg/l

of glutamic acid in the basal medium to culture *Gyrodinium cohnii.*[1] (Gold and Baren, p. 256). Glutamic acid is an amino acid and therefore, is a carboxylic acid. WO 01/04338 states that "[p]referably, the carbon source is a carboxylic acid." (p. 14) The reference also meets the limitation in Embodiment A of "the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid." *Gyrondinium cohnii* is well known (and acknowledged by WO 01/04338) to produce docosahexaenoic acid. Accordingly, Gold and Baren anticipates Embodiment A.

II. Droop

This reference discloses "culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group." Droop teaches acetate as the main carbon source for growth of the algae *Euglena gracilis* and for the dinoflagellate *Oxyrrhis marina.* (Droop, p. 533 and Table 19.1, p. 534). Acetate is recognized in WO 01/04338 as a preferred embodiment of an "ionized form of an acidic group." (p. 8) The reference also meets the limitation in Embodiment A of "the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid." It is known in the art that both *Oxyrrhis marina* and *E. gracilis* synthesize docosahexaenoic acid.[2] Accordingly, Droop anticipates Embodiment A.

III. Provasoli and Gold

This reference discloses "culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group." The Provasoli and Gold reference teaches acetate as the main carbon source for growth of *Gyrodinium cohnii*[3]. (Provasoli and Gold, Table 1,

---

[1] *Gyrodinium cohnii* was subsequently re-named *Crypthecodium cohnii.* Brands, S.J. (Comp.) 1989-2004. *Systema Naturae 2000.* Amsterdam, The Netherlands.

[2] See, *e.g.*, Meyer A et al., Biosynthesis of docosahexaenoic acid in *Euglena gracilis*: biochemical and molecular evidence for the involvement of a delta-4 fatty acyl group desaturase, *Biochemistry* 42(32):9779-88; Buckmaster, A. (http:www.mtsu.edu/~mcnair/abstracts/abuckmaster 02.pdf)(summer 2002); and Klein Breteler, WCM et al. (1999) Trophic upgrading of food quality by protozoans enhancing copepod growth, Marine Biology, 135(1):191-98.

[3] *Gyrodinium cohnii* was subsequently re-named *Crypthecodium cohnii.* Brands, S.J. (Comp.) 1989-2004. *Systema Naturae 2000.* Amsterdam, The Netherlands.

p. 198 and p. 199) Acetate is recognized in WO 01/04338 as a preferred embodiment of an "ionized form of an acidic group." (p. 8) In addition, this reference identifies a number of acids as carbon sources for *G. cohnii*, namely acetic acid, propionic acid, butyric acid, valeric acid, caproic acid, heptylic acid, and caprylic acid. (Provasoli and Gold, p. 199) The reference also meets the limitation in Embodiment A of "the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid." *Gyrodinium cohnii* is well known (and acknowledged by WO 01/04338) to produce docosahexaenoic acid. Accordingly, the Provasoli and Gold reference anticipates Embodiment A.

III. Provasoli

This reference discloses "culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group." Provasoli teaches that *Crypthecodinium cohnii* can utilize acetic acid as a carbon source. (Provasoli, p. 1302) The reference also meets the limitation in Embodiment A of "the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid." *C. cohnii* is well known (and acknowledged by WO 01/04338) to produce docosahexaenoic acid. Accordingly, Provasoli anticipates Embodiment A.

IV. Hastings and Thomas

This reference discloses "culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group." The Hastings and Thomas reference teaches that *Gyrodinium cohnii*[4] can utilize acetic acid as a carbon source. (Hastings and Thomas, p. 116) The reference also meets the limitation in Embodiment A of "the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid." *C. cohnii* is well known (and acknowledged by WO 01/04338) to produce docosahexaenoic acid. Accordingly, this reference anticipates Embodiment A.

V. Long (WO 89/00606)

---

[4]*Gyrodinium cohnii* was subsequently re-named *Crypthecodium cohnii.* Brands, S.J. (Comp.) 1989-2004. *Systema Naturae 2000.* Amsterdam, The Netherlands

This reference discloses "culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group." The Long reference teaches inclusion of 0.2 g sodium glutamate in growth media for *Crypthecodinium cohnii*. (Long, Example 3, p. 7) Glutamate is the ionized form of glutamic acid, a carboxylic acid. WO 01/04338 states that "[p]referably the carbon source is a carboxylic acid." (p. 14) The reference also meets the limitation in Embodiment A of "the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid." *C. cohnii* is well known (and acknowledged by WO 01/04338) to produce docosahexaenoic acid. Accordingly, Long anticipates Embodiment A.

VI. Barclay (U.S. 5,130,242)

This reference discloses "culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group." Barclay teaches inclusion of glutamate into growth media for *Thraustochytrium* and *Schizochytrium* microorganisms. (See Barclay, Table 7, col. 31, ll. 50, disclosing glutamate as a media component) Glutamate is the ionized form of glutamic acid, a carboxylic acid. WO 01/04338 states that "[p]referably the carbon source is a carboxylic acid." (p. 14) The reference also meets the limitation in Embodiment A of "the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid." Barclay teaches that *Thraustochytrium* and *Schizochytrium* produce docosahexaenoic acid (Barclay, Tables 3-4, col. 23-27). Accordingly, Barclay anticipates Embodiment A.

VII. Bahnweg

This reference discloses"culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group." Specifically, Bahnweg teaches use of glutamate for culturing Thraustochytrids. (Bahnweg Table 1, p. 271). Glutamate is the ionized form of glutamic acid, a carboxylic acid. WO 01/04338 states that "[p]referably the carbon source is a carboxylic acid." (p. 14) The reference also meets the limitation in Embodiment A of "the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid." It is

known in the art (*e.g.*, U.S. 5,130,244) that Thraustochytrids produce docosahexaenoic acid. Accordingly, Bahnweg anticipates Embodiment A.

VIII. Henderson, Leftley, and Sargent

This reference discloses "culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group." Henderson teaches use of a radiolabeled acetate into *Crypthecodinium cohnii* culture to follow its incorporation into fatty acids. (Henderson, p. 1681) Acetate is recognized by WO 01/04338 as a preferred embodiment of an "ionized form of an acidic group." (p. 8) The reference also meets the limitation in Embodiment A of "the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid," since the reference demonstrates radiolabeled acetate incorporated into fatty acid chains, thus showing that the acetate is serving as a carbon source (Henderson, p. 1680-1). WO 01/04338 acknowledges that C. *cohnii* produces docosahexaenoic acid. Accordingly, Henderson anticipates Embodiment A.

IX. Beach

This reference discloses "culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group." Beach teaches use of a radiolabeled acetate into *Crypthecodinium cohnii* culture to follow its incorporation into fatty acids. (Beach, p. 16) Acetate is recognized by WO 01/04338 as a preferred embodiment of an "ionized form of an acidic group." (p. 8) The reference also meets the limitation in Embodiment A of "the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid," since the reference demonstrates radiolabeled acetate incorporated into fatty acid chains, thus showing that the acetate is serving as a carbon source. (Beach, p. 16) WO 01/04338 acknowledges that *C. cohnii* produce docosahexaenoic acid. Accordingly, Beach anticipates Embodiment A.

X. Henderson and Mackinlay

This reference discloses "culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group." Henderson and Mackinlay teach use of a radiolabeled acetate into *Crypthecodinium cohnii* culture to follow its incorporation into fatty acids.

(Henderson, p. 1781) Acetate is recognized by WO 01/04338 as a preferred embodiment of an "ionized form of an acidic group." (p. 8) The reference also meets the limitation in Embodiment A of "the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid," since the reference demonstrates radiolabeled acetate incorporated into fatty acid chains, thus showing that the acetate is serving as a carbon source. (Henderson, p. 1781) WO 01/04338 acknowledges that *C. cohnii* produce docosahexaenoic acid. Accordingly, Henderson and Mackinlay anticipate Embodiment A.

XI. Bajpai

This reference discloses "culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group." Bajpai teaches culturing *Thraustochytrium aureum* with glutamate. (Bajpai, Table 1, p. 707) Glutamate is the ionized form of glutamic acid, a carboxylic acid. WO 01/04338 states that "[p]referably the carbon source is a carboxylic acid." (p. 14). The reference also meets the limitation in Embodiment A of "the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid." The Bajpai reference discloses production of docosahexaenoic acid by the organism under these conditions. (Bajpai, p. 708). Accordingly, Bajpai anticipates Embodiment A.

XII. Kyle (EP 0 515 460)

This reference discloses "culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group." Kyle teaches culturing *Crypthecodinium cohnii* with yeast extract (which contains glutamate). (Kyle, col. 4, ll. 26-29) The reference also meets the limitation in Embodiment A of "the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid." Kyle discloses production of docosahexaenoic acid by the organism under these conditions. (Kyle, col. 4, ll. 26-29) Accordingly, Kyle anticipates Embodiment A.

Embodiment A(1)–the method of Embodiment A, wherein the organism is a dinoflagellate or genetically modified variant thereof.

The following references meet all the limitations of Embodiment A, (*i.e.*, culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group, the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid) and specifically disclose dinoflagellates. These references are characterized in the above section entitled "Embodiment A." Specifically, those references include Gold and Baren (*C. cohnii*); Droop (*Oxyrrhis marina*); Provasoli and Gold (*C. cohnii*); Provasoli (*C. cohnii*); Hastings and Thomas (*C. cohnii*); Long (WO 89/00606)(*C. cohnii*); Henderson, Leftley, and Sargent (*C. cohnii*); Beach (*C. cohnii*); and Henderson and Mackinlay (*C. cohnii*).

Embodiment A(2)–the method of Embodiment A(1), wherein the microorganism is *Crypthecodinium cohnii* or a genetically modified variant thereof.

The following references meet all the limitations of Embodiment A, (*i.e.*, culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group, the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid) and specifically disclose *C. cohnii*. These references are characterized in the above section entitled "Embodiment A." Specifically, those references include Gold and Baren; Provasoli and Gold; Provasoli; Hastings and Thomas; Long (WO 89/00606); Henderson, Leftley, and Sargent; Beach; and Henderson and Mackinlay.

Embodiment A(3)–the method of Embodiment A, wherein the species is a carboxylic acid or carboxylic ion.

The following references meet all the limitations of Embodiment A, (*i.e.*, culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group, the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid) and specifically disclose using organic acids containing a carboxylic acid or a carboxylic ion. Such organic acids/ions include glutamate, acetate, propionate, butyrate, valerate, and so on. These references are characterized in the above section entitled "Embodiment A." Specifically, those references include Gold and Baren (glutamate); Droop (acetate); Provasoli and Gold (acetate);

Provasoli (acetate, succinate, fumarate); Hastings and Thomas (acetate); Long (WO 89/00606) (glutamate); Barclay (glutamate); Bahnweg (glutamate); Henderson, Leftley, and Sargent (acetate); Beach (acetate); and Henderson and Mackinlay (acetate).

Embodiment A(4)–the method of Embodiment A, wherein the species is acetic acid or acetate.

The following references meet all the limitations of Embodiment A, (*i.e.*, culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group, the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid) and specifically disclose using acetic acid or acetate. These references are characterized in the above section entitled "Embodiment A." Specifically, those references include Droop (acetate); Provasoli and Gold (acetate); Provasoli (acetate); Hastings and Thomas (acetate); Henderson, Leftley, and Sargent (acetate); Beach (acetate); and Henderson and Mackinlay (acetate).

Embodiment A(5)–the method of Embodiment A, wherein the species is the main carbon source for the microorganism during the culture of the microorganism.

The following references meet all the limitations of Embodiment A, (*i.e.*, culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group, the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid) and specifically disclose the species being the main carbon source for the microorganism. These references are characterized in the above section entitled "Embodiment A." Specifically, those references include Provasoli and Gold (p. 199); Provasoli (p. 1302); Bahnweg (p. 271); and Hastings and Thomas (p. 116).

Embodiment A(6)–the method of Embodiment A, wherein the microorganism is cultured in a medium, an amount of the species being provided in the medium over a period of time during the culture of the microorganism.

The following reference meets all the limitations of Embodiment A, (*i.e.*, culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic

group, the microorganism using the species as a carbon source and synthesizing docosahexaenoic acid) and specifically disclose the amount of the species provided in the medium over a period of time during the culture of the microorganism. This reference is characterized in the above section entitled "Embodiment A." Specifically, Barclay, col. 9, ll. 37-41 discloses the addition of acid over the course of the fermentation to stabilize the pH.

Embodiment A(7)–the method of Embodiment A(6), wherein the use of the species as a carbon source by the microorganism causes an increase in pH of the medium, said provision of the species comprising addition of an organic acid to the medium in response to the increase in pH so as to decrease the pH of the medium.

The following reference meets all the limitations of Embodiment A(6), and specifically discloses the addition of an organic acid to the medium to decrease the pH of the medium. This reference is characterized in the above section entitled "Embodiment A." Specifically, Barclay, col. 9, ll. 37-41, discloses that the culture medium typically becomes more alkaline during the fermentation and that pH may be controlled by the addition of acids (an acid may be either organic or inorganic).

Embodiment A(8)–the method of Embodiment A(7), wherein the organic acid is the species.

As noted above, the Barclay reference meets all the limitations of Embodiment A(7), and specifically discloses the species being an organic acid. As known in the art, in aqueous solution, at lower pH organic acids will tend to be in the acidic form rather than the salt (*i.e.*, ionized) form. The Barclay reference, alone or in combination with the following references, teaches the elements of Embodiment A(8). Gold and Baren (glutamic acid/glutamate); Droop (acetic acid/acetate); Provasoli and Gold (acetic acid/acetate); Provasoli (acetic acid/acetate); Hastings and Thomas (glutamic acid/glutamate); Long (WO 89/00606) (glutamic acid/glutamate); Barclay (glutamic acid/glutamate); Bahnweg (glutamate); Henderson, Leftley, and Sargent (acetic acid/acetate); Beach (acetic acid/acetate); and Henderson and Mackinlay (acetic acid/acetate). These references are characterized in the above section entitled "Embodiment A."

Embodiment A(9)–the method of Embodiment A(7), wherein the organic acid ionizes to form the species.

As noted above, the Barclay reference meets all the limitations of Embodiment A(7), and specifically discloses the species being an ionized organic acid. As known in the art, in aqueous solution, at higher pH organic acids will tend to be in the salt (*i.e.*, ionized) form rather than the acidic form. The Barclay reference, alone or in combination with the following references, teaches the elements of Embodiment A(9): Gold and Baren (glutamic acid/glutamate); Droop (acetic acid/acetate); Provasoli and Gold (acetic acid/acetate); Provasoli (acetic acid/acetate); Hastings and Thomas (glutamic acid/glutamate); Long (WO 89/00606) (glutamic acid/glutamate); Barclay (glutamic acid/glutamate); Bahnweg (glutamate); Henderson, Leftley, and Sargent (acetic acid/acetate); Beach (acetic acid/acetate); and Henderson and Mackinlay (acetic acid/acetate). These references are characterized in the above section entitled "Embodiment A."

Embodiment A(10)–the method of Embodiment A(7), wherein the organic acid is added so as to maintain the pH substantially at a desired value.

The following reference meets all the limitations of Embodiment A(7), and specifically discloses the addition of an organic acid to the medium to maintain the pH substantially at a desired value. This reference is characterized in the above section entitled "Embodiment A." Specifically, Barclay, col. 9, ll. 37-41, discloses that the culture medium typically becomes more alkaline during the fermentation and that pH may be maintained at a desired value by the addition of acids (an acid may be either organic or inorganic).

Embodiment A(11)–the method of Embodiment A(7), wherein the desired value is pH 6.5.

The following reference meets all the limitations of Embodiment A(7), and specifically discloses the addition of an organic acid to the medium to maintain the pH at a desired value. This reference is characterized in the above section entitled "Embodiment A." Specifically, Barclay, col. 9, ll. 37-41, discloses that the culture medium typically becomes more alkaline during the

fermentation and that pH may be maintained at a desired value (preferably 5.5-8.5) by the addition of acids (an acid may be either organic or inorganic).

Embodiment A(12)–the method of Embodiment A(7), wherein the pH of the medium is monitored by means that produces a signal that is used to control the addition of the organic acid to the medium.

The Barclay reference, characterized in the above section entitled "Embodiment A," meets all the limitations of Embodiment A(7). The further limitation of Embodiment A(12) of monitoring the pH to produce a signal to control acid addition is merely automation of a known process. Such automation is an obvious mode of implementation of the disclosure in Barclay, col. 9, ll. 37-41 of controlling the pH by acid addition over the course of the fermentation.

Embodiment A(13)–the method of Embodiment A(12), wherein the signal is used to control addition of one or more of a nitrogen source, a phosphorous source, an amino acid, a vitamin, a salt or another growth factor during the culture of the microorganism.

The Barclay reference discloses or suggests all the limitations of Embodiment A(12). The further limitation of Embodiment A(13) of addition of nutrient components during culturing is standard procedure during continuous culturing, as disclosed in Barclay, col. 10, ll. 36-40, and as such is an obvious mode of implementing the Barclay disclosure.

Embodiment A(14)–the method of Embodiment A(7), wherein the organic acid is added to the medium in a mixture comprising a further compound.

The Barclay reference discloses all the limitations of Embodiment A(7) and discloses addition of both acids and of additional nutrients. This reference is characterized in the above section entitled "Embodiment A." Specifically, Barclay, col. 9, ll. 37-41 discloses acid addition and col. 10, ll. 36-41 discloses nutrient addition by disclosing continuous fermentation over the course of the fermentation. The addition of acid in a mixture comprising a further compound is an obvious mode of implementing the Barclay disclosure.

Embodiment A(15)–the method of Embodiment A(14), wherein the further compound is a further organic acid.

The Barclay reference discloses or suggests all the limitations of Embodiment A(14) and specifically discloses the addition of an organic acid to the medium to decrease the pH of the medium. This reference is characterized in the above section entitled "Embodiment A." Specifically, Barclay, col. 9, ll. 37-41, discloses that the culture medium typically becomes more alkaline during the fermentation and that pH may be controlled by the addition of acid (an acid may be either organic or nonorganic). The phrase "pH control by acid addition" refers to pH control by adding compounds with an acid function, not to addition of a single species of acid. Therefore, this phrase would be understood by one of skill in the art to refer to more than one species of acid, including an additional or "further" species of acid.

Embodiment A(16)–the method of Embodiment A(14), wherein the further compound is a lipid.

The Barclay reference discloses or suggests all the limitations of Embodiment A(14) and discloses addition of both acids and of additional nutrients. This reference is characterized in the above section entitled "Embodiment A." Specifically, Barclay, col. 9, ll. 37-41 discloses acid addition and col. 10, ll. 36-41 discloses nutrient addition by disclosing continuous fermentation over the course of the fermentation. The addition of lipid in a mixture comprising a further compound is an obvious mode of implementing the Barclay disclosure.

Embodiment A(17)–the method of Embodiment A(14), wherein the mixture is a waste product from an industrial process

The Barclay reference discloses or suggests all the limitations of Embodiment A(14) and discloses addition of both acids and of additional nutrients. This reference is characterized in the above section entitled "Embodiment A." Specifically, Barclay, col. 9, ll. 37-41 discloses acid addition and col. 10, ll. 36-41 discloses nutrient addition by disclosing continuous fermentation over

the course of the fermentation. The use of a waste product from an industrial process as a nutrient source is an obvious mode of implementing the Barclay disclosure.

Embodiment A(18)–the method of Embodiment A(14), wherein the further compound is a nitrogen source, a phosphorus source, an amino acid, a vitamin, a growth factor, a salt or a lipid.

The Barclay reference discloses or suggests all the limitations of Embodiment A(14) and discloses addition of both acids and of additional nutrients. This reference is characterized in the above section entitled "Embodiment A." Specifically, Barclay, col. 9, ll. 37-41 discloses acid addition and col. 10, ll. 36-41 discloses nutrient addition by disclosing continuous fermentation over the course of the fermentation. Nutrients as disclosed by Barclay include a nitrogen source, a phosphorus source, amino acids and vitamins, among other nutrients. See Barclay, col. 19, ll. 61 through col. 20, ll 36.

Embodiment A(19)–the method of Embodiment A, wherein prior to said culturing with said species, the microorganism is grown with said species.

The Barclay reference meets all the limitations of Embodiment A and specifically discloses that prior to culturing with the species, the microorganism is grown with the species. This reference is characterized in the above section entitled "Embodiment A." Specifically, Barclay, Example 2, col. 19, l. 61 through col. 20, l. 36, discloses an initial culturing with yeast extract (which contains glutamate), followed by seeding another culture with 1 milliliter of this initial culture.

Embodiment A(20)–the method of Embodiment A, wherein the microorganism is cultured with an organic nitrogen source, preferably with yeast extract.

The Bajpai, Kyle and Barclay references each meet all the limitations of Embodiment A and specifically disclose that the microorganism is cultured with an organic nitrogen source, preferably with yeast extract. Specifically, Bajpai, Table 1, p. 707; Kyle, col. 6 ll. 10-12; and Barclay, Example 2, col. 19 l. 61 through col. 20, l. 36, all disclose culturing a microorganism with yeast extract to produce docosahexaenoic acid.

Embodiment A(21)–the method of Embodiment A(20), wherein the nitrogen source is yeast extract and the initial concentration of the yeast extract is greater than 7.5 g/l.

The Bajpai reference meets all the limitations of Embodiment A(20) and disclose that the microorganism is cultured with an organic nitrogen source, preferably with yeast extract. Specifically, Bajpai, Table 1, p. 707 discloses culturing a microorganism with yeast extract (up to 10 g/l) to produce docosahexaenoic acid.

Embodiment A(22)–the method of Embodiment A(20), wherein the initial concentration of yeast extract is 10 g/l.

The Kyle reference meets all the limitations of Embodiment A(20)and discloses that the microorganism is cultured with an organic nitrogen source, preferably with yeast extract. Specifically, Kyle, col. 6, ll. 10-12, discloses culturing a microorganism with yeast extract (from 8 to 15 g/l) to produce docosahexaenoic acid.

Embodiment A(23)–the method of Embodiment A, wherein the microorganism is cultured with salts or osmoticants, preferably with sea salts.

The following references meet all the limitations of Embodiment A and specifically disclose that the microorganism is cultured with salts or osmoticants, preferably with sea salts. Specifically, Bajpai, Table 1, p. 707 teaches use of a salt mixture (NaCl, MgSO4, and KCL, which approximates that of seawater. Barclay (Examples 1, 5, and 9) teaches the use of seawater, diluted seawater, and artificial seawater in the cultivation of DHA producing microorganisms. Kyle teaches the use of seawater or artificial seawater (col. 4, l. 54 to col. 5, l. 6). Provasoli and Gold teach cultivation of DHA producing organisms in a seawater-like salts medium (Table 1, p.198).

Embodiment A(24)–the method of Embodiment A, wherein said culture is performed as a batch process or a fed-batch process.

The Kyle reference meets all the limitations of Embodiment A and specifically discloses that the culture is performed as a fed-batch process. Specifically, Kyle (col. 5, ll. 47-55 and Examples 1 and 2) discloses fed-batch culturing of *Crypthecodinium cohnii*.

Embodiment A(25)–the method of Embodiment A(24), wherein the culture is performed for between about 4 to 10 days, preferably between about 6 to about 9 days.

The Kyle reference meets all the limitations of Embodiment A(24), and discloses a fed-batch culture performed for between about 4 to 10 days. Specifically, Kyle, Fig. 3, discloses continuous culturing for about 6.7 days.

Embodiment A(26)–the method of Embodiment A, wherein said culture is performed as a continuous process or a semi-continuous process.

The Barclay and Kyle references meet all the limitations of Embodiment A and disclose that the culture is performed as a continuous or semi-continuous process. Specifically, Barclay, col. 10, ll. 36-40 and Kyle, col. 8, l. 55 through col. 10, l. 28, disclose continuous culturing.

Embodiment A(27)–the method of Embodiment A, wherein the method further comprises extracting oil including docosahexaenoic acid from the microorganism and, preferably, purifying the oil to increase the docosahexaenoic acid content of the oil.

The following references meet all the limitations of Embodiment A, and extracting oil including docosahexaenoic acid from the microorganism. Specifically, the following references disclose extraction of DHA oil: Bajpai (p. 707); Kyle (col. 7, ll. 29-58); Barclay (col. 13, l. 41 through col. 14, l. 47); and Long (p. 5, ll. 3-12).

Embodiment A(28)–the method of Embodiment A(27), wherein the method further comprises the purification or partial purification of docosahexaenoic acid from the microorganism.

The following references meet all the limitations of Embodiment A(27), and purifying or partially purifying docosahexaenoic acid from the microorganism. Specifically, the following references disclose purifying or partially purifying DHA oil: Bajpai (p. 707); Kyle (col. 7, ll. 29-58); Barclay (col. 13, l. 41 through col. 14, l. 47); and Long (p. 5, ll. 3-12).

Embodiment A(29)–the method of Embodiment A, wherein the culture does not include a stationary phase.

The Barclay and Kyle references meet all the limitations of Embodiment A and disclose continuous culturing. Barclay (col. 10, ll. 36-40) and Kyle (col. 8, l. 55 through col. 10, l. 28). Continuous cultures are understood to be in the exponential phase of growth, and not in a stationary phase.

Embodiment A(30)–an oil comprising docosahexaenoic acid prepared from a microorganism cultured in accordance with Embodiment A.

The following references meet all the limitations of Embodiment A. Specifically, the following references disclose a DHA oil prepared by methods of Embodiment A: Bajpai (p. 707); Kyle (col. 7, ll.29-58; Barclay (col. 13, l. 41 through col. 14, l. 47); and Long (p. 5, ll. 3-12).

Embodiment A(31)–an at least partially purified preparation of docosahexaenoic acid prepared from a microorganism cultured in accordance with Embodiment A.

The following references meet all the limitations of Embodiment A. Specifically, the following references disclose a partially purified docosahexaenoic acid prepared by methods of Embodiment A: Bajpai (p. 707); Kyle (col. 7, ll.29-58; Barclay col. 13, l. 41 through col. 14, l. 47); and Long (p. 5, ll. 3-12).

Embodiment A(32)–the method of Embodiment A, wherein the initial concentration of the species is about 8 g/l.

The following references meet all the limitations of Embodiment A: Gold and Brown, Droop, Provasoli and Gold; Provasoli; Hastings and Thomas; Long; Barclay; Bahnweg; Henderson, Leftley and Sargent; Beach; Henderson and Mackinlay; Bajpai; and Kyle. The use of a species concentration of about 8 g/l is an obvious mode of implementing these references.

Embodiment B–a method of culturing a microorganism for the synthesis of docosahexaenoic acid by the microorganism, comprising culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group, the microorganism synthesizing docosahexaenoic acid containing carbon from the species.

The following references meet all the limitations of Embodiment B by disclosing culturing the microorganism with an organic species, where the microorganism synthesizes docosahexaenoic acid containing carbon from the species: Gold and Baren (glutamate); Droop (acetate); Provasoli and Gold (acetate); Provasoli (acetate, succinate, fumarate); Hastings and Thomas (glutamate); Long (WO 89/00606) (glutamate); Barclay (glutamate); Bahnweg (glutamate); Henderson, Leftley, and Sargent (acetate); Beach (acetate); and Henderson and Mackinlay(acetate). Particularly, Henderson, Leftley and Sargent; Beach; and Henderson and Mackinlay explicitly demonstrate that docosahexaenoic acid synthesized by the microorganism incorporates carbon from acetate.

Embodiment C–a method of culturing a microorganism for the synthesis of a polyunsaturated fatty acid by the microorganism, comprising culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group, the microorganism using the species as a carbon source and synthesizing a polyunsaturated fatty acid.

The following references meet all the limitations of Embodiment C by disclosing culturing the microorganism with an organic species, where the microorganism synthesizes a polyunsaturated fatty acid (docosahexaenoic acid) where the microorganism uses the species as a carbon source: Gold and Baren; Droop; Provasoli and Gold; Provasoli; Hastings and Thomas; Long; Barclay; Bahnweg; Henderson, Leftley, and Sargent; Beach; and Henderson and Mackinlay.

Embodiment C(1)–an oil comprising the polyunsaturated fatty acid of Embodiment C, prepared from a microorganism cultured in accordance with Embodiment C.

The following references meet all the limitations of Embodiment C, and therefore disclose a polyunsaturated fatty acid (*i.e.*, docosahexaenoic acid) prepared by methods of Embodiment C: Bajpai (p. 707); Kyle (col. 7, ll.29-58); Barclay (col. 13, l. 41 through col. 14, l. 47); and Long (p. 5, ll. 3-12).

Embodiment C(2)–an at least partially purified preparation of the polyunsaturated fatty acid of Embodiment C, prepared from a microorganism cultured in accordance with Embodiment C.

The following references meet all the limitations of Embodiment C, and therefore, disclose or suggest an at least partially purified polyunsaturated fatty acid preparation (*i.e.*, docosahexaenoic acid) prepared by methods of Embodiment C: Bajpai (p. 707); Kyle (col. 7, ll.29-58); Barclay (col. 13, l. 41 through col. 14, l. 47); and Long (p. 5, ll. 3-12).

Embodiment C(3)–a method comprising using a microorganism according to Embodiment C as a food source.

The following references meet all the limitations of Embodiment C, including using a microorganism according to Embodiment C as a food source: Kyle (col. 7, ll. 41-48); Barclay (col. 14, ll. 48-54); and Long (p. 5, ll. 3-12).

Embodiment D–a microorganism cultured in accordance with any one of Embodiments A, B, or C.

The following references meet all the limitations of Embodiment D. The following references disclose a microorganism, which is cultured in accordance with any of the embodiments of Embodiments A, B, or C. Specifically, the following references disclose such organisms: Gold and Baren; Droop; Provasoli and Gold; Provasoli; Hastings; Long; Barclay; Henderson, Leftley, and Sargent; Beach; and Henderson and Mackinlay.

Obviousness–35 U.S.C. 103

Obviousness under 35 U.S.C. § 103(a) is found if "the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious to a person having ordinary skill in the art to which said subject matter pertains." 35 U.S.C. § 103(a). The claimed Embodiment must be considered as a whole; the cited references must suggest the desirability and thus the obviousness of making the combination; references must be viewed without impermissible hindsight; and a reasonable expectation of success is the standard by which obviousness is determined. Hodosh v. Block Drug Co., 786 F.2d 1136, 1143 n. 5, 229 USPQ 182, 187 n. 5 (Fed. Cir. 1986).

Embodiments A-D

To the extent that the references used above for anticipation arguments are construed as not completely anticipatory, these references render Embodiments A-D obvious.

Specifically, methods to culture microorganisms and obtain docosahexaenoic acids, polyunsaturated fatty acids, and microorganisms in accordance with Embodiments A-D are obvious under 35 U.S.C. 103(a) the references discussed above either alone or in view the other references. Specifically, it would be obvious to use the methods of culturing with an organic species comprising an acidic group or an ionized form of an acidic group, to culture an microorganism capable of producing docosahexaenoic acid or a polyunsaturated fatty acid, to produce a docosahexaenoic acid or polyunsatured fatty acid.

In summary, the instant patent application(s) to methods of culturing a microorganism for the synthesis of polyunsaturated fatty acid, especially docosahexaenoic acid, comprising culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group, the microorganism using the species as a carbon source and synthesizing polyunsaturated fatty acid, especially docosahexaenoic acid, to oils produced by the methods, and to microorganisms produced by the methods. In view of the art presented above, rendering this subject matter anticipated and/or obvious, the Examiner is respectfully requested to reject each and every one of the presently pending claims in the instant application(s) as anticipated and/or obvious over the cited prior art.

Respectfully submitted,

SHERIDAN ROSS P.C.

By: _____

Gary J. Connell
Registration No. 32,020
1560 Broadway, Suite 1200
Denver, Colorado 80202-5141
(303) 863-9700

Date: January 28, 2005